Reference: *Biol. Bull.* **197:** 7–10. (August 1999)

Mechanical Resistance to Shear Stress: The Role of Echinoderm Egg Extracellular Layers

FLORENCE I. M. THOMAS[1,2,*,†], KRISTEN A. EDWARDS[1,2], TOBY F. BOLTON[1,2,*],
MARY A. SEWELL[3], AND JILL M. ZANDE[1]

[1] *The Marine Environmental Sciences Consortium, Dauphin Island Sea Lab, Dauphin Island, Alabama;*
[2] *The Department of Marine Sciences, The University of South Alabama, Mobile, Alabama; and*
[3] *The Department of Biology, University of Southern California, Los Angeles, California*

Extracellular layers (jelly coats) on echinoderm eggs are composed of a fibrous network imbedded in a gelatinous material. This type of fibrous network has the potential to protect eggs from mechanical stress. To determine the effects of shear stress and the role of jelly coats in protecting eggs from these stresses, eggs of the sea urchin Lytechinus variegatus, *both with and without intact jelly coats, were exposed to shear stresses ranging from 0.3 to 2 Pa in a cone and plate viscometer. The percentage of eggs remaining intact after exposure to the shear stress was assessed. The results indicate that shear stress can damage eggs and that jelly coats may play a role in decreasing the effects of these stresses. Eggs with jelly coats remained intact and fertilizable at greater shear stresses than those with the coats removed. This is the first evidence that extracellular layers on invertebrate eggs can provide protection from mechanical forces.*

In free-spawning invertebrates, the eggs and sperm, having passed through a gonoduct and gonopore, are released directly into the water column. During the spawning process, gametes are exposed to shear stresses (force per area) in the gonoduct and in the external environment. If gametes are harmed by shear stress before fertilization, irreversible damage will effectively lower the probability of fertilization by decreasing the number of viable gametes in a volume of seawater. Moreover, if gametes are damaged by shear stress, they may survive and be fertilized, but the resultant embryos may not develop normally. Therefore, the number of larvae produced by a given number of eggs may be reduced by exposure to shear stress.

Eggs are first exposed to shear stresses as they traverse the gonoduct on their way to the gonopore. As fluid moves down a tube such as the oviduct, a shear gradient develops in the fluid and imposes a shear stress on the fluid and eggs within the duct. Estimates of shear stresses in the oviduct of one species of sea urchin, *Arbacia punctulata,* ranged from near zero at the center of the duct to over 41 Pa near the wall of the duct (1). This magnitude of shear stress exceeds that estimated to occur in the external environment (2), where gametes meet their second challenge from shear stress after they are released from the gonopore. After eggs are released, they encounter shear stress produced by the interaction of eddies within the water column or within the momentum boundary layer at the surface of the spawning adult. The effects of such shear stress on fertilization and development have only recently been addressed in a hydrodynamic study of fertilization in the purple sea urchin *Strongylocentrotus purpuratus* (2). High shear stress produced experimentally in a Couette cell resulted in low fertilization success and abnormal embryo development. Mead and Denny (2) postulate that high shear stress, as produced in turbulent environments, might reduce the encounter rates of gametes during fertilization, decrease the ability of sperm to remain attached to the egg after contact, and damage eggs prior to fertilization and embryos after fertilization.

Given the two sources of shear stress that eggs experience prior to fertilization, it is possible that there has been selection for egg properties that could reduce the damage caused by these stresses. This idea is supported by the fact that at least one property of echinoid eggs, their viscosity, is positively correlated to wave exposure in three species (3). One

Received 16 April 1998; accepted 2 June 1999.

* Present address: Department of Biology, University of South Florida, Tampa, Florida 33620-5150

† To whom correspondence should be addressed. E-mail: fthomas@chuma1.cas.usf.edu

property of eggs that has the potential to decrease the effects of shear stress is the extracellular layer, or jelly coat, that encapsulates the eggs of echinoderms (4). These coats are composed of a fibrous network imbedded in globular glycoprotein (5) and can account for a significant portion of the maternal energy invested in an egg (Bolton and Thomas, unpubl. data). Jelly coats are described for both echinoids and asteroids and consist of several concentric layers of complex fibrous networks (5–10) that are reminiscent of engineering materials designed to withstand shear stresses (11, 12). Although the jelly coat in echinoderms is involved in many parts of the fertilization process (13–18), it seems unlikely that this complex network is required for any of these functions. Thus it is possible that the structure of jelly coats plays a role in protecting eggs from shear stresses experienced in the external environment after spawning, in the oviduct during spawning, or both. The purpose of the research presented here is to explore whether jelly coats can provide this protection in the sea urchin *Lytechinus variegatus*.

To examine the potential role of jelly coats in resisting shear stress, we exposed eggs with and without jelly coats to shear stresses in a cone and plate viscometer and determined the percentage of those eggs that remained intact and fertilizable. A greater percentage of eggs with jelly coats remained intact after exposure to shear stress than did those with jelly coats removed (Fig. 1, Table I). A test for homogeneity of slopes indicated that shear stress affected eggs with jelly coats differently than eggs with coats removed. Eggs with jelly coats suffered a maximum loss of less than 4% as shear stresses approached 2 Pa. No eggs were damaged until shear stresses in excess of 1.7 Pa were reached. For eggs without jelly coats, damage occurred at lower shear stresses than for those with coats, and maximum loss reached nearly 20% as shear stress approached 2 Pa.

The fertilization success of eggs exposed to shear decreased with increasing shear stress both for eggs with jelly coats and for those without (Fig. 2). For both egg types, fertilization success was near 85% with no shear (the de-

Table I

Effect of shear stress on percentage of eggs remaining intact

Source	*F*	*P*
With/without coats (same intercepts)	1.99	0.165
Shear stress (slope = 0)	0.20	<0.001
Interaction (same slopes)	0.75	<0.001

Results from a test for homogeneity of slopes (1 degree of freedom) performed on the arcsine-transformed percentage of eggs intact after exposure to shear stress *versus* the magnitude of shear stress for eggs with and without jelly coats. There was a significant effect of shear stress on eggs remaining intact (slope is significantly different from 0). Eggs with and without jelly coats were affected by shear stress to a different degree (significant interaction between shear stress and jelly coat presence).

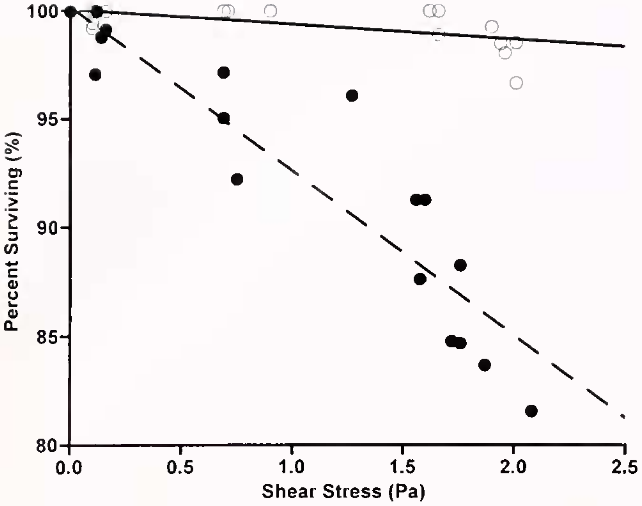

Figure 1. Percentage of *Lytechinus variegatus* eggs remaining intact after exposure to shear stresses. Sea urchins were collected from St. Joseph's Bay, Florida, transported to the Dauphin Island Sea Lab, Alabama, and maintained in laboratory aquaria. Additional animals were obtained from the Carolina Biological Supply Company. Urchins were maintained in seawater collected from St. Joseph Bay (salinity 29–30 ppt), aerated continuously, and fed either spinach or lettuce. We obtained eggs by injecting urchins with 0.5 M KCl. Eggs were used immediately after spawning. Paired samples of eggs with and without jelly coats from each of five females were prepared by removing the jelly coats from half of the eggs collected from a female. The coats were removed using a technique commonly employed in embryology (19). Eggs were poured through a plankton screen (Nytex) with 202-μm-diameter pores. To determine whether the jelly coats had been removed from these eggs, a 200-μl aliquot was pipetted onto a depression slide and Sumi ink was used to visualize the edges of the jelly coats under a compound microscope at 100 × magnification (20). To minimize the probability of damaging the eggs by subjecting them to unnecessary passes through the plankton screen, the eggs were checked for the presence of jelly coats after every two pours.

To assess the effects of shear stress and the potential effect of jelly coats on egg survival, eggs were exposed to a range of shear stresses in a cone and plate viscometer (Brookfield Digital Viscometer, DV-II) attached to a constant temperature bath (Neslab RTE-8). Shear stress was changed by adjusting the shear rate and the viscosity of the fluid (μ). The viscosity was altered by adding hydroxyethyl cellulose (Proto-slo) to filtered seawater. Eight viscous fluids were tested: KY jelly (chlorhexidine gluconate); hydroxyethyl cellulose; polyvinylpyrrolidone; polyvinylpolypyrrolidone; Percoll; methylcellulose; Dextran; and egg homogentate. Two criteria were used to select a fluid: (1) after exposure to the fluid, eggs both with and without jelly coats remained viable; and (2) no cell leakage was detected with visual inspection. Hydroxyethyl cellulose was the only fluid that met both criteria, so it was chosen for use in the experiment. Experiments at shear stresses up to 1.5 Pa were also conducted in seawater only, and the results indicated that use of the hydroxyethyl cellulose could not account for all of the egg loss.

A known number of eggs in 0.5 ml filtered seawater were exposed to a given shear stress for 2 min. The shear stress significantly affected the survival of eggs with (open circles: arcsine % intact = 1.57–0.07* shear stress, $r^2 = 0.48$, $P < 0.001$; 95% confidence limits for the slope are 0.04 to 0.10) and without jelly coats (closed circles: arcsine % intact = 1.53–0.28* shear stress, $r^2 = 0.91$, $P < 0.0001$, 95% confidence limits for the slope are 0.24 to 0.32). For those with jelly coats, there was little effect of shear stress: percent survival ranged from 100% to 96.7% over the entire range of shear stresses. For those without jelly coats, the effect of shear stress was more apparent: percent survival ranged from 100% to 82.0%.

sired initial fertilization success). As shear increased, fertilization success dropped from 85% to 68% for eggs with coats and from 85% to 59% for eggs without coats. A test for homogeneity of slopes indicated that the shear stress affected fertilization differently for eggs without intact coats than for those with coats present (Fig. 2, Table II). The slope of the regression of fertilization *versus* shear stress was steeper for eggs with jelly coats removed than for those with intact coats (Fig. 2). After fertilization, developing embryos were assessed for developmental stage and normal development up to the pleuteus stage. Development was assessed throughout this period. All fertilized eggs, regardless of whether they had jelly coats or not, developed normally. This result indicates that if eggs are damaged by shear stress they are not fertilizable, but if they survive they apparently have not sustained any damage that limits normal development.

Table II

Effect of shear stress on fertilization

Source	*F*	*P*
With/without coats (same intercepts)	2.6	0.13
Shear stress (slope = 0)	0.001	<0.001
Interaction (same slopes)	5.90	0.032

Results from a test for homogeneity of slopes (1 degree of freedom) performed on the arcsine-transformed percentage of eggs fertilized after exposure to shear stress *versus* the magnitude of shear stress for eggs with and without jelly coats. There was a significant effect of shear stress on fertilization (slope is significantly different from 0). Eggs with and without jelly coats were affected by shear stress to a different degree (significant interaction between shear stress and jelly coat presence).

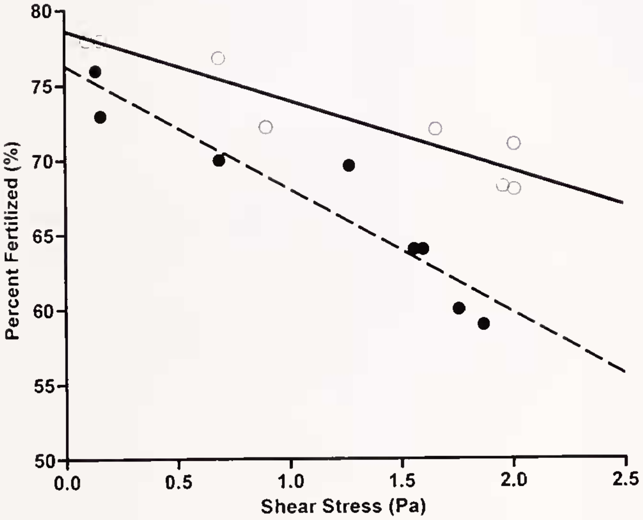

Figure 2. Percentage of eggs successfully fertilized after exposure to shear stress. The fertilizability of eggs with and without intact jelly coats was determined after eggs were exposed to a given shear for 2 min. Gametes for all experiments were obtained by injecting urchins with 0.5 *M* KCl. Females were allowed to spawn into filtered seawater; males spawned into a dry petri dish. Sperm were stored undiluted in a scintillation vial over ice until being used in the experiments. Eggs were used immediately after spawning and sperm within 1 h after spawning. The concentration of sperm yielding 80%–85% successful fertilization (10^4 dilution of dry spawned sperm in seawater) was determined from serial dilution experiments. We used this sperm dilution in all fertilization experiments to ensure that any fertilization decrease caused by egg damage would not be concealed by an overabundance of sperm (2). After exposure to shear, the eggs were added to 100 ml of seawater with the sperm solution. Un-sheared eggs were also fertilized as a control. A sample of approximately 60–100 eggs was examined for fertilization success. Fertilization was assessed at the four-cell stage of development, and normal development was determined after embryos reached the early pluteus stage.

There was a negative relationship between fertilization success and exposure to shear stress, both for eggs with jelly coats (open circles: arcsine % fertilized = 0.92–0.07* shear stress, r^2 = 0.88, $P < 0.001$, 95% confidence limits for the slope are −0.04 to −0.09) and for those without (closed circles: arcsine % fertilized = 0.87–0.11* shear stress, r^2 = 0.90, $P < 0.001$, 95% confidence limits for the slope are −0.08 to −0.15). Only eggs exposed to shear in the viscometer were used in these analyses. Results of a test for homogeneity of slopes indicates that shear stress affects fertilization success of eggs with jelly coats less than that of those without jelly coats (Table II).

The results of these experiments indicate that eggs can be damaged by shear stress and that the jelly coats on echinoderm eggs can provide mechanical strength, reducing the negative effects of shear stress on free-spawned eggs. If jelly coats are absent, survivorship (Fig. 1) and fertilization success (Fig. 2) are significantly less than when coats are present.

The shear regime experienced in the viscometer most closely approximates that seen in the gonoduct. The experimental shear is unidirectional, constant, and well within the range estimated to occur in the gonoduct during spawning (1). In contrast, the shear stresses imposed in the external environment not only are lower (2) than those used in these experiments, but are not constant and are unlikely to be unidirectional for sustained periods of time. Thus, the data presented here indicate strongly that eggs are susceptible to shear stress and can be damaged at stress levels in the range experienced during egg release. Therefore, the data show that jelly coats on echinoderm eggs do protect eggs from damage caused by shear stress. This result is significant because it is the first evidence that extracellular layers on invertebrate eggs can protect eggs from physical stress and may provide mechanical strength to eggs.

Acknowledgments

Research support was provided by a National Science Foundation (NSF) grant (IBN-9723770) and an NSF PECASE award to F. I. M. Thomas (OCE-9701434). Kristen Edwards initiated the research while participating in a research experience for undergraduate program funded by a NSF Grantto Judy Stout (REU, OCE-9619862). We also thank three anonymous reviewers for helpful suggestions. This is DISL contribution 309.

Literature Cited

1. **Thomas, F. I. M., and T. F. Bolton. In Press.** Shear stress experienced by echinoderm eggs in the oviduct during spawning: potential role in the evolution of egg properties. *J. Exp. Biol.*
2. **Mead, K. S., and M. W. Denny. 1995.** The effects of hydrodynamic shear stress on fertilization and early development of the purple sea urchin *Strongylocentrotus purpuratus*. *Biol. Bull.* **188:** 46–56.
3. **Thomas, F. I. M. 1994.** Physical properties of gametes in three sea urchin species. *J. Exp. Biol.* **194:** 263–284.
4. **Hoshi, M. 1985.** Lysins. Pp. 431–462 in *Biology of Fertilization*, Vol. 2. C. B. Metz and A. Monroy, eds. Academic Press, Orlando, FL.
5. **Bonnell, B. S., S. H. Keller, V. D. Vacquier, and D. E. Chandler. 1994.** The sea urchin jelly coat consists of globular glycoproteins bound to a fibrous fucan superstructure. *Dev. Biol.* **162:** 313–324.
6. **Kidd, P. 1978.** The jelly and vitelline coats of the sea urchin egg: new ultrastructural features. *J. Ultrastruct. Res.* **64:** 204–215.
7. **Holland, N. D. 1980.** Electron microscopic study of the cortical reaction in eggs of the starfish (*Patiria miniata*). *Cell Tissue Res.* **205:** 67–76.
8. **Crawford, B., and M. Abed. 1986.** Ultrastructural aspects of the surface coatings of eggs and larvae of the starfish, *Pisaster ochraceus*, revealed by Alcian Blue. *J. Morphol.* **187:** 29–37.
9. **Sousa, M., R. Pinto, P. Moradas-Ferreira, and C. Azevedo. 1993.** Histochemical studies of jelly coat of *Marthasterias glacialis* (Echinodermata, Asteroidea) oocytes. *Biol. Bull.* **185:** 215–224.
10. **Bonnell, B. S., C. Larabell, and D. E. Chandler. 1993.** The sea urchin egg jelly coat is a three-dimensional fibrous network as seen by intermediate voltage electron microscopy and deep etching analysis. *Mol. Reprod. Dev.* **35(2):** 181–188.
11. **Sastry, A. M., S. L. Phoenix, and P. Schwartz. 1993.** Analysis of interfacial failure in a composite microbundle pull-out experiment. *Composite Sciences and Technology.* **48:** 237–251.
12. **Sastry, A. M., X. Cheng, and C. W. Wang. 1998.** Mechanics of stochastic fibrous networks. *J. Thermoplastic Composite Materials. Vol. II.* 288–296.
13. **Vaquier, V. D., and G. W. Moy. 1977.** Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs. *Proc. Natl. Acad. Sci. USA* **74:** 2456–2460.
14. **Tilney, L. G., D. P. Kiehart, C. Sardet, and M. Tilney. 1978.** Polymerization of actin IV. Role of $Ca2^+$ and H^+ in the assembly of actin and in membrane fusion in the acrosomal reaction of echinoderm sperm. *J. Cell Biol.* **77:** 536–550.
15. **SeGall, G. K., and W. J. Lennarz. 1981.** Jelly coat and induction of the acrosome reaction in echinoid sperm. *Dev. Biol.* **86:** 87–93.
16. **Garbers, D. L., and G. S. Kopf. 1980.** The regulation of spermatazoa by calcium and cyclic nucleotides. *Adv. Cyclic Nucleotide Res.* **13:** 251–306.
17. **Nomura, K., and S. Isaka. 1985.** Synthetic study of the structure-activity relationship of sperm-activating peptides from the jelly coat of sea urchin eggs. *Biochem. Biophys. Res. Commun.* **126:** 974–982.
18. **Miller, R. L. 1985.** Sperm chemo-orientation in the Metazoa. Pp. 276–337 in *Biology of Fertilization*, Vol. 2. C. B. Metz and A. Monroy, eds. Academic Press, Orlando, FL.
19. **Hinegardner, R. 1975.** Care and handling of sea urchin eggs, embryos, and adults (principally North American species). Pp. 10–41 in *The Sea Urchin Embryo—Biochemistry and Morphogenesis*. G. Czihak, ed. Springer-Verlag, New York.
20. **Strathmann, M. F. 1987.** *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast.* University of Washington Press, Seattle. 670 pp.